

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Patent Examining Operations

06/09/00
1c837 U.S. PRO

Applicant(s): Adamou and Choi

Serial No: Unassigned

Art Unit:

Filed: Herewith

Examiner: Unassigned

TITLE: STREPTOCOCCUS PNEUMONIAE PROTEINS AND VACCINES

Docket No: 469201-475

1c530 U.S. PRO
09/590991
06/09/00

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

Application Transmittal Letter

Dear Sir:

Transmitted herewith for filing is the patent application of:

John E. Adamou and Gil H. Choi

entitled: STREPTOCOCCUS PNEUMONIAE PROTEINS AND VACCINES

Enclosed with the application are:

1. Application (42 sheets), Drawings (informal; 4 sheets); Sequence Listing (10 sheets);
2. Declaration of Adamou and Choi (unexecuted);
3. Statement under 37 C.F.R. 1.821(f);
4. Sequence Listing in computer readable form (3.5" diskette);
5. Postage paid, return receipt postcard; and
6. Our check number 2590 in the amount of \$882.00.

The fee has been calculated as follows:

	<u>Number of</u> <u>Claims Filed</u>			<u>Extra</u> <u>Claims</u>	<u>Rate</u>	<u>Fee</u>
Basic Fee					\$690	\$690
Total Claims	22	-	20 =	2	\$ 18	\$ 36
Indep. Claims	5	-	3 =	2	\$ 78	\$156
TOTAL:						\$882

The Commissioner is authorized to charge payment of any additional filing fees required under 37 CFR 1.16 associated with this communication or credit any overpayment to Deposit Account No. 03-0678. A duplicate copy of this paper is enclosed.

EXPRESS MAIL CERTIFICATE

Express Mail Label No. EL290883214US

Deposit Date: 9 June 2000

I hereby certify that this paper and the attachments hereto are being deposited today with the U.S. Postal Service "Express Mail Post Office To Addressee" service under 37 CFR 1.10 on the date indicated above addressed to:

**BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, DC 20231**


Alan J. Grant, Esq.

6/9/00
Date

Respectfully submitted,



Alan J. Grant, Esq.
Reg. No. 33,389
CARELLA, BYRNE, BAIN, GILFILLAN,
CECCHI, STEWART & OLSTEIN
6 Becker Farm Road
Roseland, NJ 07068
Tel. No.: (973) 994-1700

***STREPTOCOCCUS PNEUMONIAE* PROTEINS AND VACCINES**

5

This application claims priority of U.S. provisional application 60/138,453, filed June 10, 1999, the disclosure of which is hereby incorporated by reference in its entirety.

10

BACKGROUND OF THE INVENTION

Streptococcus pneumoniae is a gram positive
15 bacterium which is a major causative agent in invasive infections in animals and humans, such as sepsis, meningitis, otitis media and lobar pneumonia (Tuomanen, et al. NEJM 322:1280-1284 (1995)). As part of the infective process, pneumococci readily bind to non-inflamed human
20 epithelial cells of the upper and lower respiratory tract by binding to eukaryotic carbohydrates in a lectin-like manner (Cundell et al., Micro. Path. 17:361-374 (1994)). Conversion to invasive pneumococcal infections for bound bacteria may involve the local generation of inflammatory
25 factors which may activate the epithelial cells to change the number and type of receptors on their surface (Cundell, et al., Nature, 377:435-438 (1995)). Apparently, one such receptor, platelet activating factor (PAF) is engaged by the pneumococcal bacteria and within a
30 very short period of time (minutes) from the appearance of PAF, pneumococci exhibit strongly enhanced adherence and

invasion of tissue. Certain soluble receptor analogs have been shown to prevent the progression of pneumococcal infections (Idanpaan-Heikkila et al., J. Inf. Dis., 176:704-712 (1997)). A number of various other proteins have been suggested as being involved in the pathogenicity of *S. pneumoniae* but only some have been confirmed as virulence factors. Despite the fact that there are capsule conjugates currently in trial, there still remains a need for identifying additional polypeptides having epitopes in common from various strains of *S. pneumoniae* in order to utilize such polypeptides as vaccines to provide protection against a wide variety of *S. pneumoniae* serotypes.

BRIEF SUMMARY OF THE INVENTION

The invention disclosed herein relates to vaccines derived from polypeptides of the pneumococcal organism *Streptococcus pneumoniae*. In accordance with the present invention there are disclosed herein several protein sequences, and fragments thereof and their corresponding nucleotide sequences used for recombinantly preparing said polypeptides.

More specifically, the present invention discloses 2 large polypeptides, one denoted Sp128 (SEQ ID NO:6), composed of 664 amino acid residues, and a second polypeptide, denoted Sp130, containing 773 amino acid residues (SEQ ID NO:8). Both Sp128 and Sp130 have been

found to confer protective properties on animals immunized with said polypeptides, or portions thereof.

5 The present invention also relates to the field of
bacterial antigens and their use, for example, as
immunogenic agents in humans and animals to stimulate an
immune response. More specifically, it relates to the
vaccination of mammalian species with one or more
10 recombinant polypeptides produced according to the
invention disclosed herein, such recombinant polypeptides
being derived from *Streptococcus pneumoniae*.

15 In accordance with the present invention, such
proteins serve as a mechanism for stimulating production
of antibodies that protect the vaccine recipient against
infection by a wide range of capsular serotypes of
pathogenic *S. pneumoniae*.

20 The invention disclosed herein further relates to
antisera and antibodies against such polypeptides useful
in diagnosis and passive immune therapy with respect to
diagnosing and treating such pneumococcal infections. Like
the vaccines disclosed herein, the antibodies specific for
such antigenic polypeptides, and fragments thereof, can be
25 prepared recombinantly by transforming cells with vectors
containing the appropriate gene sequences to produce the
active tetrameric antibody. Such methods are well known in
the art.

30 In a particular aspect, the present invention relates
to the prevention and treatment of pneumococcal infections
such as infections of the middle ear, nasopharynx, lung

and bronchial areas, blood, CSF, and the like, that are caused by pneumococcal bacteria.

The present invention further relates to vaccines prepared from the novel proteins and polypeptides, as well as fragments and segments thereof, disclosed herein. In addition, examples of the use of such proteins and polypeptides as vaccines for the protection of mammals are likewise disclosed.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the results of 2 experiments (Figs 1A and 1B, respectively), using the same preparations of Sp128 and Sp130 polypeptides. The results demonstrate that active immunization with recombinant Sp128 or Sp130 polypeptides derived from the pneumococcal strain Norway serotype 4 is able to protect mice from death in a model of pneumococcal sepsis using the heterologous strain SJ2 (serotype 6B). In these 2 experiments, 90% and 100%, respectively, of the mice immunized with Sp130 survived the 14 day observation period following challenge with about 400 CFU (colony forming units) of pneumococci. Conversely, 100% of sham immunized mice (injected only with PBS (phosphate-buffered saline) plus adjuvant) died during the same period. In addition, for both experiments, 90% of the mice immunized with Sp128 survived the same 14 day observation period.

Figure 2 shows the results of passive administration of rabbit antiserum raised against Sp130 derived from Norway serotype 4. Such administration was able to protect mice in the pneumococcal sepsis model using a heterologous strain. More specifically, 70% of the mice immunized with the Sp130 antiserum survived the 10 day observation period after challenge with 1400 CFU of strain WU2 (serotype 3). In addition, 100% of the mice immunized with a control serum (collected before immunization) died by day 4.

10

Figure 3 is a western blot showing reactivity of antisera raised against recombinant Sp130 (derived from strain Norway serotype 4) with whole cell lysates of heterologous strains. All *S. pneumoniae* strains tested showed a band of molecular weight about 220 kD, the expected mass for a protein containing both the Sp128 and Sp130 sequences, indicating that this protein was present in all of the tested strains. Tested strains included isolates from each of the pneumococcal serotypes represented in the currently used 23-valent polysaccharide vaccine.

15

20

Figure 4 is a western blot showing the reactivity of patient sera with either Sp128 or Sp130. Fig. 4A shows the results for Sp128. Fig. 4B shows the results for Sp130. The recombinant proteins were resolved by SDS-PAGE and transferred to nitrocellulose. Sera were collected from 5 patients (indicated by number at the top) at two different times. First collection (denoted "A" for "acute serum") was soon after onset of illness; second collection (denoted "C" for "convalescent") was made 8 to 30 days later. These sera were used to probe the blots. The

25

30

results show that for patients 2, 3 and 5, convalescent serum reacted more strongly with Sp128 and Sp130 than did the corresponding acute serum. Such findings constitute indirect evidence that both Sp128 and Sp130 are expressed
5 by *S. pneumoniae* during this phase of infection.

DETAILED SUMMARY OF THE INVENTION

10

In accordance with the present invention there is disclosed herein recombinant polypeptides corresponding to Sp128 (SEQ ID NO: 6) and Sp130 (SEQ ID NO: 8).

15

It is an object of the present invention to provide methods of utilizing these recombinant polypeptides, and immunogenically active fragments thereof, as a means of immunizing animals, especially mammals, most especially
20 humans, against a variety of microbial infections, especially pneumococcal infections.

It is a further object of the present invention to provide polypeptides, as disclosed herein, and active
25 fragments thereof, whether derived from natural sources or prepared by means of recombinant technology, for use in immunizing animals, especially mammals, most especially humans, against pneumococcal infection.

30 It is a still further object of the present invention to provide vaccines that include polypeptides obtained from *S. pneumoniae* and/or variants of said polypeptides

and/or active fragments of such polypeptides, including polypeptides prepared by recombinant means (i.e., recombinant polypeptides and proteins).

5 In accordance with the present invention, there are also disclosed herein nucleic acids and DNA sequences and molecules, and fragments thereof (and their corresponding isolated RNA sequences, and molecules and fragments thereof) showing sequence homology with, or identity to,
10 or capable of hybridizing to, the DNA sequences identified in SEQ ID NOS: 5 and 7. The present invention also relates to DNA (or RNA) sequences encoding the same polypeptide as is encoded by the sequences of SEQ ID NOS: 5 and 7, including fragments and portions thereof and, when derived
15 from natural sources, including alleles thereof, for the express purpose of facilitating the recombinant expression of the immunogenic polypeptides, and immunogenic fragments thereof, disclosed herein.

20 Thus, an isolated DNA (or RNA) sequence can include only the coding region of the expressed gene (or fragment or portion thereof as hereinabove indicated) or can further include all or a portion of the non-coding DNA (or RNA) of the expressed human gene.

25 In accordance with the present invention, the term "percent identity" or "percent identical," when referring to a sequence, means that a sequence is compared to a claimed or described sequence after alignment of the
30 sequence to be compared (the "Compared Sequence") with the described or claimed sequence (the "Reference Sequence"). The Percent Identity is then determined according to the

following formula:

$$\text{Percent Identity} = 100 [1 - (C/R)]$$

5

wherein C is the number of differences between the Reference Sequence and the Compared Sequence over the length of alignment between the Reference Sequence and the Compared Sequence wherein (i) each base or amino acid in
10 the Reference Sequence that does not have a corresponding aligned base or amino acid in the Compared Sequence and (ii) each gap in the Reference Sequence and (iii) each aligned base or amino acid in the Reference Sequence that is different from an aligned base or amino acid in the
15 Compared Sequence, constitutes a difference; and R is the number of bases or amino acids in the Reference Sequence over the length of the alignment with the Compared Sequence with any gap created in the Reference Sequence also being counted as a base or amino acid.

20

If an alignment exists between the Compared Sequence and the Reference Sequence for which the percent identity as calculated above is about equal to or greater than a specified minimum Percent Identity then the Compared
25 Sequence has the specified minimum percent identity to the Reference Sequence even though alignments may exist in which the hereinabove calculated Percent Identity is less than the specified Percent Identity.

30

In accordance with the present invention, there are disclosed herein the polynucleotide sequences coding for the polypeptide vaccines of the invention so as to

facilitate recombinant expression of said polypeptides. Such polynucleotides code for the polypeptides of SEQ ID NOS: 6 and 8 and are disclosed as the sequences of SEQ ID NOS: 5 and 7.

5

For purposes of recombinantly expressing the polypeptide vaccines of the invention, the polynucleotides of SEQ ID NOS: 5 and 7 may also have the coding sequence fused in frame to a marker sequence which allows for
10 purification of the polypeptide of the present invention. The marker sequence may be a hexa-histidine tag (for example, as can be supplied by a pQE-9 vector) to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or, for example,
15 the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

20

To facilitate generation of the polynucleotides disclosed herein, appropriate PCR primers are provided as SEQ ID NOS: 1 (5'-primer for Sp128), 2 (3'-primer for Sp128), 3 (5'-primer for Sp130), and 4 (3'-primer for
25 Sp130).

The polypeptides, and fragments thereof, of the vaccines disclosed as expression products according to the invention may be in "enriched form." As used herein, the
30 term "enriched" means that the concentration of the material is at least about 2, 5, 10, 100, or 1000 times its natural concentration (for example), advantageously

0.01%, by weight, preferably at least about 0.1% by weight. Enriched preparations of about 0.5%, 1%, 5%, 10%, and 20% by weight are also contemplated. The sequences, constructs, vectors, clones, and other materials
5 comprising the present invention can advantageously be in enriched or isolated form.

"Isolated" in the context of the present invention with respect to polypeptides means that the material is
10 removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living organism is not isolated, but the same polypeptide, separated from some or all of the co-existing
15 materials in the natural system, is isolated. Such polypeptides could be part of a composition, and still be isolated in that such composition is not part of its natural environment. The polypeptides of the vaccines disclosed herein are preferably provided in an isolated
20 form, and preferably are purified to homogeneity.

The recombinant or immunogenic polypeptides disclosed in accordance with the present invention may also be in "purified" form. The term "purified" does not require
25 absolute purity; rather, it is intended as a relative definition, and can include preparations that are highly purified or preparations that are only partially purified, as those terms are understood by those of skill in the relevant art. For example, individual clones isolated from
30 a cDNA library have been conventionally purified to electrophoretic homogeneity. Purification of starting material or natural material to at least one order of

magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. Furthermore, claimed polypeptide which has a purity of preferably 0.001%, or at least 0.01% or 0.1%;
5 and even desirably 1% by weight or greater is expressly contemplated.

The term "coding region" refers to that portion of a gene which either naturally or normally codes for the
10 expression product of that gene in its natural genomic environment, i.e., the region coding *in vivo* for the native expression product of the gene. The coding region can be from a normal, mutated or altered gene, or can even be from a DNA sequence, or gene, wholly synthesized in the
15 laboratory using methods well known to those of skill in the art of DNA synthesis.

The term "primer" means a short nucleic acid sequence that is paired with one strand of DNA and
20 provides a free 3'OH end at which a DNA polymerase starts synthesis of a deoxyribonucleotide chain.

At the simplest level, the amino acid sequence corresponding to all or part of the polypeptides according
25 to the present invention can be synthesized using commercially available peptide synthesizers. This is particularly useful in producing small peptides and fragments of larger polypeptides. (Fragments are useful, for example, in generating antibodies against the native
30 polypeptide.)

The terms "fragment," "derivative" and "analog," when

referring to the polypeptides according to the present invention, means a polypeptide which retains essentially the same biological function or activity as said polypeptide. Thus, an analog includes a proprotein which
5 can be activated by cleavage of the proprotein portion to produce an active mature polypeptide. Such fragments, derivatives and analogs must have sufficient similarity to the polypeptides SEQ ID NOS: 6 and 8, so that activity of the native polypeptide is retained.

10

The polypeptide vaccines of the present invention may be recombinant polypeptides, natural polypeptides or synthetic polypeptides, preferably recombinant polypeptides.

15

"Recombinant," as used herein, means that a protein is derived from recombinant (e.g., microbial or mammalian) expression systems. "Microbial" refers to recombinant proteins made in bacterial or fungal (e.g., yeast)
20 expression systems. As a product, "recombinant microbial" defines a protein essentially free of native endogenous substances and unaccompanied by associated native glycosylation. Protein expressed in most bacterial cultures, e.g., E. coli, will be free of glycosylation
25 modifications that might normally occur in yeast or mammalian expression systems. Thus, the patterns of such post-translational modifications will differ with the expression system. However, all such variants are considered to lie within the disclosure of the present
30 invention.

A vaccine according to the present invention would

include a polypeptide, including immunogenic fragments thereof, comprising an amino acid sequence at least 65% identical, preferably 80% identical, most preferably 95% identical and ideally 100% identical to the amino acid sequence of SEQ ID NO:6.

Such vaccines would also comprise a polypeptide, including immunogenic fragments thereof, having an amino acid sequence at least 65% identical, preferably 80% identical, most preferably 95% identical, and ideally 100% identical to the amino acid sequence of SEQ ID NO:8.

The present invention is also directed to an antiserum produced by immunizing an animal with a polypeptide according to the invention. The invention also includes and isolated antibody that binds specifically to a polypeptide of the invention. Such an antibody may be a monoclonal antibody, possibly produced by a hybridoma cell line, and may also include a recombinantly produced antibody formed by introducing into a suitable cell line the gene sequences required for producing an antibody specific for the polypeptide vaccines disclosed herein.

The present invention is also directed to a vaccine comprising one or more *S. pneumoniae* polypeptides selected from the polypeptides, and immunogenic fragments thereof, disclosed herein, suspended in a pharmaceutically acceptable diluent, carrier or excipient, provided that said polypeptide is present in an amount effective to elicit protective antibodies in an animal against an organism related to the genus *Streptococcus*, preferably an organism of the genus *Streptococcus*, and most preferably

where the organism is *Streptococcus pneumoniae*.

The present invention also provides for a method of preventing or treating an infection caused by a member of the genus *Streptococcus* in an animal, comprising administering to an animal, especially a mammal, and most especially a human being, a polypeptide, or immunogenic fragment thereof, as disclosed herein, and wherein said polypeptide, or immunogenic fragment thereof, is administered in an amount effective to prevent or attenuate said infection. In using the methods of the invention, the disease to be prevented or treated will preferably be a pneumococcal infection, most preferably an infection by an organism that is a member of the genus *Streptococcus*, ideally *Streptococcus pneumoniae*.

A vaccine disclosed according to the present invention may also include a vaccine comprising a microbial organism transformed with polynucleotides, and thereby expressing the polypeptides, or fragments thereof, selected from the group consisting of Sp128 and Sp130 (SEQ ID NOS: 6 and 8, respectively). The present invention would thus also encompass a method of preventing or attenuating an infection caused by a member of the genus *Streptococcus* in an animal, especially a mammal, most especially a human, comprising administering to said animal such a vaccine, wherein said vaccine is administered in an amount effective to prevent or attenuate said infection. In applying the method of the invention, the transformed microorganism is selected from the group consisting of Salmonella, Mycobacteria, Streptococcus, poxviruses, and adenoviruses.

Fragments or portions of the polypeptides of the present invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length polypeptides. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.

10 The immunogenic fragments of the polypeptide vaccines disclosed according to the invention will include immunogenic fragments of Sp128 (SEQ ID NO:6), which fragments can be readily screened for immunogenic activity, as well as immunogenic fragments of Sp130 (SEQ ID NO: 8). For example, in the amino acid sequence of Sp130, the fragment corresponding to residues 657 through 773 are known to provide about 40% protection versus the entire Sp130 sequence. Thus, the former fragment protects about 4 out of 10 mice challenged with *Streptococcus pneumoniae* versus 10 of 10 for the entire Sp130 sequence. Thus, specific fragment may include the fragments having amino acid sequences 650 - 773, 640 - 773, 630 - 773, 620 - 773, 610 - 773, 600 - 773, and similar fragments up to the entire Sp130 sequence (SEQ ID NO: 8). It is logical to presume that fragments of Sp128 (SEQ ID NO: 6) may provide similar degree of protection versus the entire Sp128 protein.

Such variations in homology for putative vaccines are well known in the art (See, for example, Hansen et al., "Active and Passive Immunity Against *Borelia bergdorferi* Decorin Binding Protein A (DbpA)," Infection and Immunity,

(May) 1998, p. 2143 - 2153; Roberts et al., "Heterogeneity Among Genes Including Decorin Binding Proteins A and B of *Borelia bergdorferi sensu lato*," Infection and Immunity, (Nov) 1998, p. 5275-5285). Such observations would
5 similarly apply to portions of the proteins disclosed herein.

Such fragments or segments find a multitude of uses. For example, such segments of the polypeptides according
10 to the present invention find use as intermediates in the synthesis of higher molecular weight structures also within the present invention.

The term "active fragment" or "immunogenic fragment"
15 means a fragment that generates an immune response (i.e., has immunogenic activity) when administered, alone or optionally with a suitable adjuvant, to an animal, such as a mammal, for example, a rabbit or a mouse, and also including a human.

20 As noted, the polypeptides, fragments or other derivatives, or analogs thereof, or cells expressing them, can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal,
25 monoclonal, chimeric, single chain, Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of polyclonal antibodies, especially where these are in the form of antisera raised against the polypeptides, or
30 fragments thereof, according to the present invention. Such antisera find use in immunization against pneumococcal infection.

Antibodies generated against a polypeptide vaccine corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptide into an animal or by administering the polypeptide to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptide itself. In this manner, even a sequence encoding only a fragment of the polypeptide can be used to generate antibodies binding the whole native polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, *Nature*, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, *Immunology Today* 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Thus, the present invention also relates to the use of the novel polypeptides disclosed herein, as well as to immunogenic fragments thereof, for the production of lymphocytes, or hybridoma cells, producing monoclonal antibodies against such polypeptides, or immunogenic fragments thereof. The present invention also relates to the hybridoma cells producing such antibodies.

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to

produce single chain antibodies to immunogenic polypeptide products of this invention.

5 The antibodies can be used in methods relating to the localization and activity of the protein sequences of the invention, e.g., for imaging these proteins, measuring levels thereof in appropriate physiological samples and the like, and for other diagnostic applications.

10 A vaccine in accordance with the present invention may include one or more of the hereinabove described polypeptides or active fragments thereof. When employing more than one polypeptide or active fragment, such as two or more polypeptides and/or active fragments may be used
15 as a physical mixture or as a fusion of two or more polypeptides or active fragments. The fusion fragment or fusion polypeptide may be produced, for example, by recombinant techniques or by the use of appropriate linkers for fusing previously prepared polypeptides or
20 active fragments.

25 In many cases, a variation in the polypeptide or active fragment is a conservative amino acid substitution, although other substitutions are within the scope of the invention.

30 In accordance with the present invention, a polypeptide variant includes variants in which one or more amino acids are substituted and/or deleted and/or inserted.

 In another aspect, the invention relates to passive

immunity vaccines formulated from antibodies against a polypeptide or active fragment of a polypeptide of the present invention. Such passive immunity vaccines can be utilized to prevent and/or treat pneumococcal infections
5 in patients. In this manner, according to a further aspect of the invention, a vaccine can be produced from a synthetic or recombinant polypeptide of the present invention or an antibody against such polypeptide.

10 As already described, another aspect the present invention relates to a method of using one or more antibodies (monoclonal, polyclonal or sera) to the polypeptides of the invention as described above for the prophylaxis and/or treatment of diseases that are caused
15 by pneumococcal bacteria. In particular, the invention relates to a method for the prophylaxis and/or treatment of infectious diseases that are caused by *S. pneumoniae*. In a still further preferred aspect, the invention relates to a method for the prophylaxis and/or treatment of otitis
20 media, nasopharyngeal and bronchial infections, and the like in humans by utilizing a vaccine of the present invention.

Generally, vaccines are prepared as injectables, in
25 the form of aqueous solutions or suspensions. Vaccines in an oil base are also well known such as for inhaling. Solid forms which are dissolved or suspended prior to use may also be formulated. Pharmaceutical carriers, diluents and excipients are generally added that are compatible
30 with the active ingredients and acceptable for pharmaceutical use. Examples of such carriers include, but are not limited to, water, saline solutions, dextrose,

or glycerol. Combinations of carriers may also be used.

Vaccine compositions may further incorporate additional substances to stabilize pH, or to function as
5 adjuvants, wetting agents, or emulsifying agents, which can serve to improve the effectiveness of the vaccine.

Vaccines are generally formulated for parenteral administration and are injected either subcutaneously or
10 intramuscularly. Such vaccines can also be formulated as suppositories or for oral administration, using methods known in the art, or for administration through nasal or respiratory routes.

The amount of vaccine sufficient to confer immunity to pathogenic bacteria is determined by methods well known to those skilled in the art. This quantity will be determined based upon the characteristics of the vaccine recipient and the level of immunity required. Typically,
15 the amount of vaccine to be administered will be determined based upon the judgment of a skilled physician. Where vaccines are administered by subcutaneous or intramuscular injection, a range of .5 to 500 μ g purified protein may be given.
20

The present invention is also directed to a vaccine in which a polypeptide or active fragment of the present invention is delivered or administered in the form of a polynucleotide encoding the polypeptide or active
25 fragment, whereby the polypeptide or active fragment is produced *in vivo*. The polynucleotide may be included in a
30

suitable expression vector and combined with a pharmaceutically acceptable carrier.

5 In addition, the polypeptides of the present invention can be used as immunogens to stimulate the production of antibodies for use in passive immunotherapy, for use as diagnostic reagents, and for use as reagents in other processes such as affinity chromatography.

10 In another aspect the present invention provides polynucleotides which encode the hereinabove described polypeptides and active fragments of the invention. The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA,
15 genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand.

Host cells are genetically engineered (transduced or
20 transformed or transfected) with the vectors comprising a polynucleotide encoding a polypeptide of the invention. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as
25 appropriate for activating promoters, selecting transformants or amplifying the polynucleotides which encode such polypeptides. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be
30 apparent to the ordinarily skilled artisan.

Vectors include chromosomal, nonchromosomal and

synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, 5 fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

10 The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

15 The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be 20 mentioned: LTR or SV40 promoter, the E. coli lac or trp, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation 25 initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably 30 contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for

eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

5 The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the proteins.

10 As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila S2 and Spodoptera Sf9; animal cells such as CHO, COS or Bowes
15 melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also
20 includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of
25 this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors
30 are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen, Inc.), pBS, pD10, phagescript, psiX174, pbluescript SK, pBS, pNH8A, pNH16a, pNH18A, pNH46A

(Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and TRP. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by

conventional peptide synthesizers.

5 Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

10 Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

15 Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the
20 late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

25 Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed
30 gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate

kinase (PGK), α -factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences.

- 5 Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

- 10 Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will
- 15 comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium
- 20 and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice, including streptococcal species, especially *S. pneumoniae*.

- 25 In a further embodiment, microbial organisms genetically transformed with polynucleotides expressing Sp128 or Sp130, or both, may themselves be used as living vaccine delivery vehicles. Examples include, but are in no way limited to, *Salmonella* species, *Mycobacterium* species,
- 30 *Streptococcus* species, poxviruses, adenoviruses, and the like. In addition, transgenic edible plants may also be

candidates for vaccine delivery.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and pGEM1 (Promega, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, a french press, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

However, preferred are host cells which secrete the polypeptide of the invention and permit recovery of the polypeptide from the culture media.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of
5 monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and
10 enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to
15 provide the required nontranscribed genetic elements.

The polypeptides can be recovered and/or purified from recombinant cell cultures by well-known protein recovery and purification methods. Such methodology may
20 include ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein
25 refolding steps can be used, as necessary, in completing configuration of the mature protein. In this respect, chaperones may be used in such a refolding procedure. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

30

The polypeptides that are useful as immunogens in the present invention may be a naturally purified product, or

a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon
5 the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated.

Procedures for the isolation of the individually
10 expressed polypeptides may be isolated by recombinant expression/isolation methods that are well-known in the art. Typical examples for such isolation may utilize an antibody to a conserved area of the protein or to a His tag or cleavable leader or tail that is expressed as part
15 of the protein structure.

Specific embodiments of the invention will now be further described in more detail in the following non-limiting examples and it will be appreciated that
20 additional and different embodiments of the teachings of the present invention will doubtless suggest themselves to those of skill in the art and such other embodiments are considered to have been inferred from the disclosure herein.

25

EXAMPLE 1

Active Protection with Anti-Sp128 and Anti-Sp130

30

A. Cloning, Expression, and Purification of Sp128 and Sp130.

The genomic DNA used as target for amplification using the polymerase chain reaction was isolated from *Streptococcus pneumoniae* (Norway strain - serotype 4), the same strain used for genomic sequencing. The nucleotide
5 sequence of the gene fragments encoding Sp128 (SEQ ID NO: 5) and Sp130 (SEQ ID NO: 7) with the corresponding amino acid sequence for polypeptides Sp128 (SEQ ID NO: 6) and Sp130 (SEQ ID NO: 8) are provided in the Sequence Listing.

10 Primers (SEQ ID NOS: 1 - 4) were designed so as to amplify either Sp128 or Sp130 gene fragments and allow their cloning into the *E. coli* expresssion vector pQE10 with, for example, subsequent expression of a histidine-tagged protein product for purification by Nickel-affinity
15 chromatography.

Thus, cloning of the fragments amplified by the primers of SEQ ID NOS: 1 and 2 results in the polypeptide of SEQ ID NO: 6 (denoted Sp128), while cloning of the
20 fragment amplified using the primers disclosed in SEQ ID NOS: 3 and 4 result in the polypeptide of SEQ ID NO: 8 (denoted Sp130).

25

B. Vaccination With Sp128 and Sp130 Results in Protection Against Lethal *S. pneumoniae* Challenge

In each of the experiments shown in Figure 1, C3H/HeJ
30 mice (10 per group) were immunized intraperitoneally (i.p.) with either Sp128 or Sp130 protein (15 µg in 50 µl

PBS (phosphate buffered saline) emulsified in 50 μ l complete Freund's adjuvant (CFA)). A group of 10 mice were sham-immunized with PBS and CFA only.

5 A second immunization of 15 μ g protein with incomplete Freund's adjuvant (IFA) was administered 3 weeks later (with the sham-immunized group receiving PBS and IFA).

10 Blood was drawn (retro-orbital bleed) at week 7. Sera from each group were pooled for analysis of anti-Sp128 and anti-Sp130 antibody by ELISA. Mice were challenged at week 8 by intraperitoneal injection of approximately 400 CFU (colony forming units) of *S. pneumoniae* strain SJ2
15 (capsular serotype 6B). In preliminary experiments, the median infection dose (LD_{50}) of this strain was determined to be approximately 10 CFU. Mice were monitored for 14 days of survival.

20 Both experiments shown in Figure 1 used the same preparations of recombinant Sp128 and Sp130.

 In the experiment shown in Figure 1A, 7-week serum collected from the 10 mice immunized with either Sp128 or
25 Sp130 each had an endpoint ELISA titer of 1:2,048,000 and 1:1,024,000, respectively. No anti-Sp128 or anti-Sp130 antibody was detected in sera from sham-immunized mice. Ninety percent of the mice immunized with either Sp128 or Sp130 protein survived the challenge (406 CFU of
30 pneumococci) for the extent of the study (14 days). One hundred percent of sham-immunized mice were dead by day 7.

In the experiment shown in Figure 1B, 7-week sera collected from the 10 mice immunized with either Sp128 or Sp130 each had an endpoint ELISA titer of 1:1,024,000 and 1:512,000, respectively. No anti-Sp128 or anti-Sp130 antibody was detected in sera from sham-immunized mice. Ninety and one hundred percent of the mice immunized with either Sp128 or Sp130 protein, respectively, survived the challenge (404 CFU of pneumococci) for the extent of the study (14 days). One hundred percent of sham-immunized mice were dead by day 5.

These data indicate that immunization of mice with either recombinant Sp128 or Sp130 proteins elicit a response capable of protecting against systemic pneumococcal infection and subsequent death. Cross protection is demonstrated by the fact that the recombinant pneumococcal protein was generated based on capsular serotype 4 DNA sequence, while the challenge was with the heterologous strain SJ2 (capsular serotype 6B).

EXAMPLE 2

Passive Protection With Anti-Sp130 Antisera

A. Generation of Rabbit Immune Sera

Following collection of pre-immune serum, a New

Zealand White rabbit was immunized with 250 µg of Sp130 (SEQ ID NO:8) in complete Freund's adjuvant. The rabbit was given 2 boosts of 125 µg Sp130 in incomplete Freund's adjuvant on days 21 and 52, and bled on days 31 and 62.

5

B. Passive Protection in Mice

BALB/cByJ mice (10 per group) were passively
10 immunized by 2 i.p. injections of 100 µl of rabbit serum. The first injection was administered 24 hours before challenge with 1400 CFU of *S. pneumoniae* strain WU2, and the second injection was given 4 hours after challenge. Figure 2 shows the survival of mice after infection with
15 WU2 (capsular serotype 3) strain. In preliminary experiments, the LD₅₀ of this strain was determined to be approximately 100 CFU.

Figure 2 shows the survival of mice injected with
20 1400 CFU of strain WU2. As shown therein, 70% of the mice immunized with rabbit immune serum raised against Sp130 protein survived the 10 day observation period. Of the mice immunized with the control serum (collected from a rabbit prior to immunization), 100% died by day 4.

25

These data suggest that the protection against pneumococcal infection resulting from immunization with Sp130 is antibody-mediated, since the mice were protected by passive transfer of serum from a hyperimmunized rabbit.
30 As seen in the previously described mouse active challenge experiments, serum directed against recombinant Sp130

protein cloned from a serotype 4 strain was protective against challenge with a heterologous strain, WU2 (capsular serotype 3).

5

10

EXAMPLE 3

15

Conservation of Sp128-Sp130 Among Strains of *S. pneumoniae*

A. Western Blotting

20

The pneumococcal strains used in this experiment were obtained from the American Type Culture Collection (Rockville, MD) and include one isolate from each of the serotypes in the currently used multivalent pneumococcal vaccine.

25

For total cell lysates, pneumococci were grown to mid-logarithmic phase (absorbance at 620 nm was 0.4 to 0.6) in 2 ml Todd-Hewitt broth with 5% yeast extract (from Difco, Detroit, MI) at 37°C. Bacteria were harvested by centrifugation and washed twice with water. Pellets (consisting of sedimented cells) were resuspended in 200

µl of lysis buffer (0.01% sodium dodecyl sulfate, 0.15 M sodium citrate, and 0.1% sodium deoxycholate) and incubated at 37°C for 30 min, then diluted in an equal volume of 2X SSC (0.3 M NaCl, 0.03 M sodium citrate).

5 Polypeptides in the lysates were resolved on SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis), transferred to nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA) and probed with antibody by conventional Western Blotting procedures. Sera
10 from a New Zealand White rabbit immunized with Sp130 (as per Example 2, supra) was used at a dilution of 1:3000. Bound antibody was detected with peroxidase-conjugated sheep anti-rabbit IgG using a chemiluminescence kit from Amersham Inc. (Cambridge, MA).

15

The rabbit anti-Sp130 sera revealed 2 major bands with apparent molecular weights of 110 kD and 220 kD in all 23 pneumococcal lysates tested (as shown in Figure 3).

20

These data show that Sp130 is expressed and shares common antigenic epitopes among strains of the 23 pneumococcal capsular serotypes represented in the currently used polysaccharide vaccine.

25

EXAMPLE 4

30 Immunogenicity of Sp128 and Sp130 in Humans

Sera from patients with culture-proven pneumococcal bacteremia were used in Western blots containing recombinant Sp128 or Sp130 protein. In the experiment shown in Figure 4, sera from 5 patients (indicated by numerals 1 through 5) were diluted 1:3000 and used to probe blots containing Sp128 (SEQ ID NO:6) or Sp130 (SEQ ID NO:8).

The lanes labeled "A" (for "acute") were probed with serum collected shortly after diagnosis of pneumococcal infection; lanes denoted "C" (for "convalescent") were probed with serum collected either 1 month later (patients 1, 2, and 3) or 8 days after the first serum collection (patients 4 and 5). For patients 2, 3, and 5, reactivity of the convalescent serum with Sp128 and Sp130 was stronger than that of the corresponding acute serum.

Other experiments (not depicted in the figure) showed that convalescent sera from 17 patients with pneumococcal infections were tested individually for reactivity with either Sp128 or Sp130. Thus, 10 and 15 of the 23 sera were found to bind (on a Western Blot) Sp128 and Sp130, respectively.

These experiments indicate that Sp128 and Sp130 (the latter to a greater extent), are recognized by the human immune system and suggest that antibodies able to bind the Sp128-Sp130 protein may be produced during natural *S. pneumoniae* infection in humans. Further, this provides indirect evidence that Sp128 and Sp130 are expressed in vivo by *S. pneumoniae* during this phase of infection, and thus may be available as targets for immunoprophylaxis,

immunotherapy, or to provide anamnestic immune responses
in subjects vaccinated with these proteins. Since the
patients were infected with a variety of pneumococcal
strains, these data also support the idea that Sp130 is
5 more antigenically conserved than Sp128.

WHAT IS CLAIMED IS:

1. A vaccine comprising a polypeptide, including immunogenic fragments thereof, having an amino acid sequence at least 65% identical to the amino acid sequence of SEQ ID NO:6.

2. The vaccine of claim 1 wherein said amino acid sequence is at least 80% identical to the amino acid sequence of SEQ ID NO:6.

3. The vaccine of claim 1 wherein said amino acid sequence is at least 95% identical to the amino acid sequence of SEQ ID NO:6.

4. The vaccine of claim 1 wherein said amino acid sequence is identical to the amino acid sequence of SEQ ID NO:6.

5. A vaccine comprising a polypeptide, including immunogenic fragments thereof, having an amino acid sequence at least 65% identical to the amino acid sequence of SEQ ID NO:8.

6. The vaccine of claim 5 wherein said amino acid sequence is at least 80% identical to the amino acid sequence of SEQ ID NO:8.

7. The vaccine of claim 5 wherein said amino acid sequence is at least 95% identical to the amino acid sequence of SEQ ID NO:8.

8. The vaccine of claim 5 wherein said amino acid sequence is identical to the amino acid sequence of SEQ ID NO:8.

5 9. An antiserum produced by immunizing an animal with a polypeptide selected from the group consisting of the polypeptides of claims 1, 2, 3, 4, 5, 6, 7, and 8.

10 10. An isolated antibody that binds specifically to a polypeptide selected from the group consisting of the polypeptides according to claims 1, 2, 3, 4, 5, 6, 7, and 8.

15 11. The antibody of claim 10 wherein the antibody is a monoclonal antibody.

12. An engineered cell producing a monoclonal antibody of claim 11.

20 13. An antiserum produced by immunizing an animal with the polypeptide of SEQ ID NO: 6.

25 14. An antiserum produced by immunizing an animal with the polypeptide of SEQ ID NO: 8.

15. An isolated recombinant antibody that binds specifically to a polypeptide selected from the group consisting of the polypeptides of claims 1,2,3,4,5,6,7, and 8.

30 16. A vaccine comprising :
a. one or more *S. pneumoniae* polypeptides selected

from the group consisting of the polypeptides of claims 1, 2, 3, 4, 5, 6, 7, and 8; and

b. a pharmaceutically acceptable diluent , carrier or excipient;

5 wherein said polypeptide is present in an amount effective to elicit protective antibodies in an animal against an organism of the genus *Streptococcus*.

10 17. A method of preventing or attenuating an infection caused by a member of the genus *Streptococcus* in an animal, comprising administering to said animal a polypeptide selected from the group consisting of the polypeptides of claims 1, 2, 3, 4, 5, 6, 7, and 8, and wherein said polypeptide is administered in an amount
15 effective to prevent or attenuate said infection.

20 18. A method of preventing pneumococcal infection by administering to an animal the vaccine according to claim 16.

25 19. A method of preventing or attenuating an infection caused by a member of the genus *Streptococcus* in an animal, comprising administering to said animal an antibody according to claim 10, wherein said antibody is administered in an amount effective to prevent or attenuate said infection.

30 20. A vaccine comprising a microbial organism transformed with polynucleotides, and thereby expressing the polypeptides, or fragments thereof, selected from the group consisting of Sp128 and Sp130.

21. A method of preventing or attenuating an infection caused by a member of the genus *Streptococcus* in an animal, comprising administering to said animal a vaccine according to claim 20, wherein said antibody is administered in an amount effective to prevent or attenuate said infection.

22. The vaccine according to claim 20, wherein said transformed microorganism is selected from the group consisting of *Salmonella*, *Mycobacteria*, *Streptococcus*, poxviruses, and adenoviruses.

15

20

25

30

ABSTRACT

The present invention relates to novel immunogenic polypeptides; and fragments thereof, and vaccines for the prevention and treatment of pneumococcal infection, especially by *Streptococcus pneumoniae*. The invention also relates to antibodies against the disclosed polypeptides, as well as vaccines containing said polypeptides and methods of disease prevention.

FIGURE 1

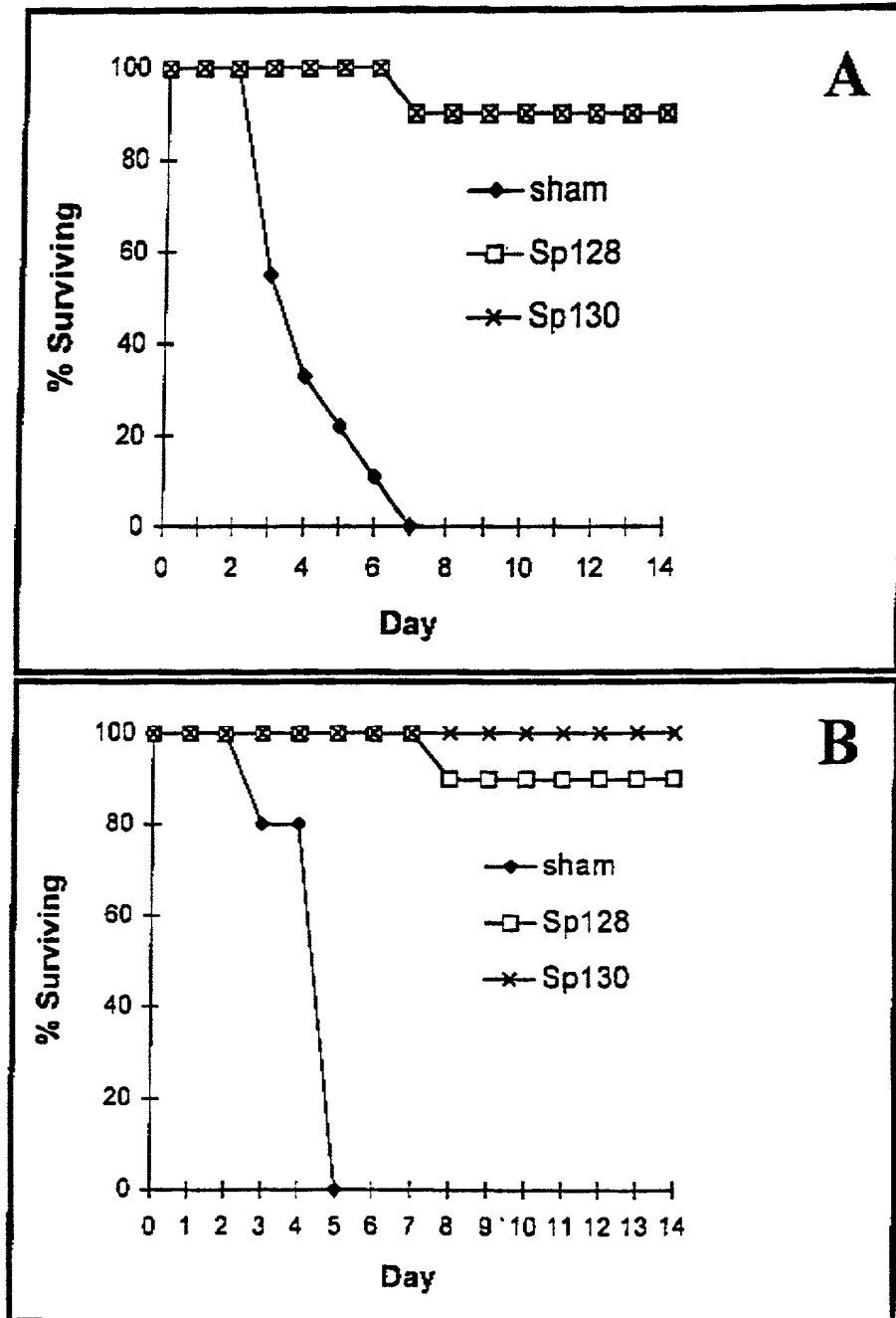
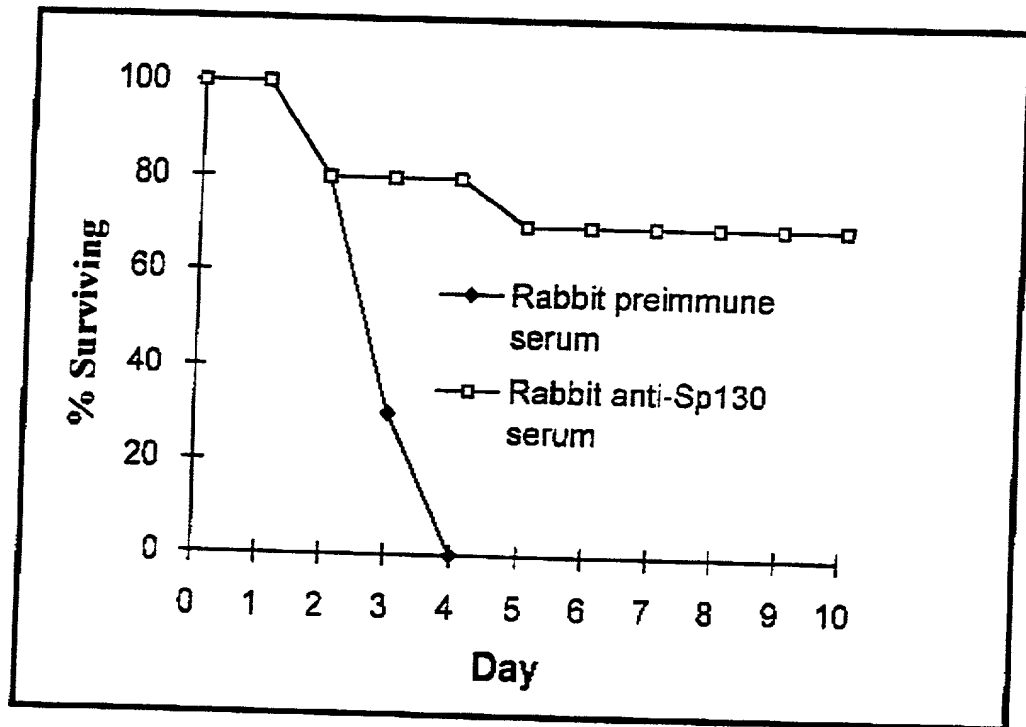


FIGURE 2

Strain WU2 (serotype 3)



3/4

FIGURE 3

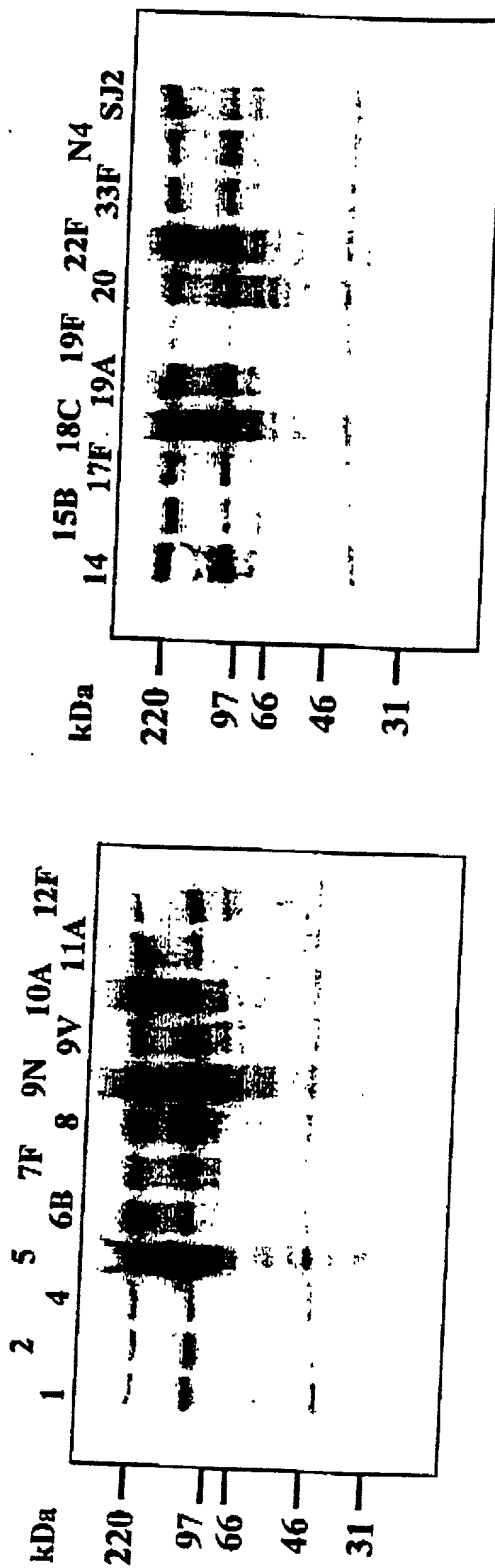
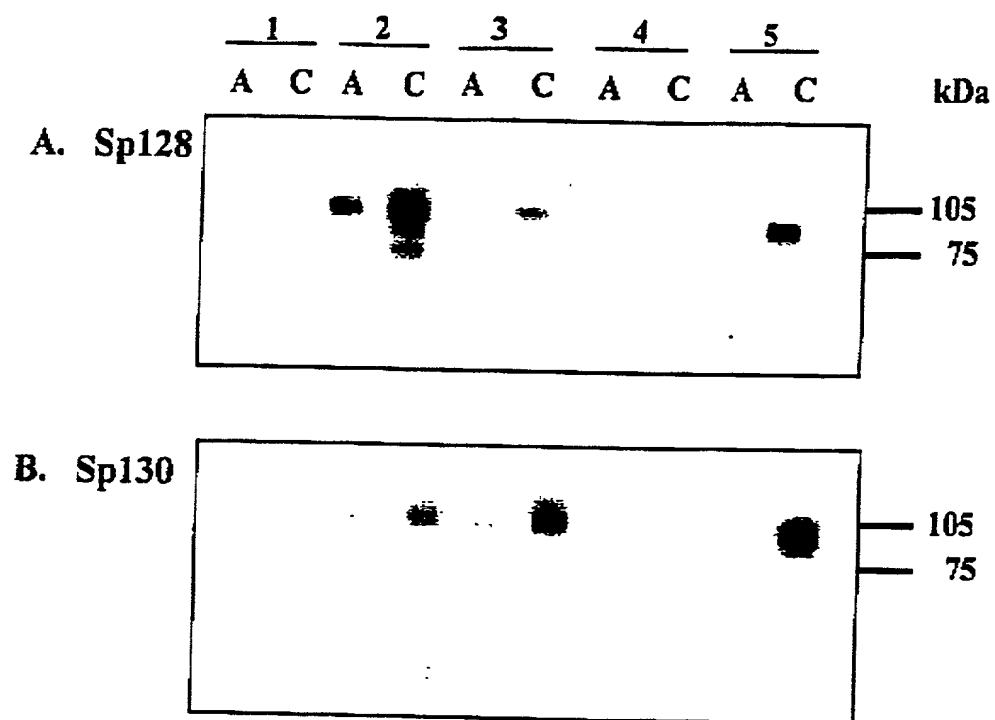


FIGURE 4



DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

STREPTOCOCCUS PNEUMONIAE PROTEINS AND VACCINES

the specification of which [X] is attached hereto or [] was filed on _____ as Application Serial No. _____ and was amended on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s):

Priority Claimed

Yes	No
<input type="checkbox"/>	<input type="checkbox"/>

(Number)

(Country)

(Day/Month/Year Filed)

I hereby claim the benefit under Title 35, United States Code, Section(s) 119 and/or 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

60/138,453

10 June 1999

Pending

(Application Serial No.)

(Filing Date)

(Status)

(Application Serial No.)

(Filing Date)

(Status – pending provisional, patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: John N. Bain (Reg. No. 18,651); John G. Gilfillan, III (Reg. No. 22,746); Elliot M. Olstein (Reg. No. 24,025); Raymond J. Lillie (Reg. No. 31,778); William Squire (Reg. No. 25,378); and Alan J. Grant (Reg. No. 33,389). Address correspondence and telephone calls to:
Alan J. Grant, Esq. c/o Carella, Byrne, Bain, Gilfillan, Cecchi, Stewart & Olstein, 6 Becker Farm Road, Roseland, NJ 07068 - (973) 994-1700.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor: John E. Adamou

Inventor's signature: _____ Date: _____
Residence: 20822 Shamrock Glen Circle, Rockville, MD 20874 Citizenship: Canada
Post Office Address: same

Full name of second joint inventor Gil H. Choi

Inventor's signature: _____ Date: _____
Residence: 11429 Potomac Oaks Drive, Rockville, MD 20850 Citizenship: Korea
Post Office Address: same

Docket No.: 469201-475

SEQUENCE LISTING

<110> Adamou, John
Choi, Gil

<120> Streptococcus Pneumoniae Proteins and Vaccines

<130> 469201-475

<140>

<141>

<150> U.S. 60/138,453

<151> 1999-06-10

<160> 8

<170> PatentIn Ver. 2.1

<210> 1

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Forward primer
for PCR amplification of Sp128 genomic sequences

<400> 1

tacccggtag tcttagcaga c

21

<210> 2

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Reverse primer
for PCR amplification of Sp128 genomic sequence

<400> 2

atagccataa gttgatttgc catta

25

<210> 3

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Forward primer
for PCR amplification of Sp130 genomic sequence

<400> 3

aagcttggcg agattgcaga a

21

<210> 4

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Reverse primer
for PCR amplification of Sp130 genomic sequence

<400> 4

cttattagga ttgttagtag ttgattt

27

<210> 5

<211> 1992

<212> DNA

<213> Streptococcus pneumoniae

<400> 5

tacccggtag tottagcaga cacatctagc totgaagatg ctttaaacad ctctgataaa 60
gaaaaagtag cagaaaataa agagaaacat gaaaatatcc atagtgtat ggaaacttca 120
caggatttta aagagaagaa aacagcagtc attaaggaaa aagaagttgt tagtaaaaat 180
cctgtgatag acaataaacac tagcaatgaa gaagcaaaaa tcaaagaaga aaattccaat 240
aaatcccaag gagattatac ggactcattt gtgaataaaa acacagaaaa tccccaaaaa 300
gaagataaag ttgtctatat tgctgaattt aaagataaag aatctggaga aaaagcaatc 360
aaggaactat ccagtcttaa gaatacaaaa gttttatata cttatgatag aatttttaac 420
ggtagtgcca tagaaacaac tccagataac ttggacaaaa ttaaacaat agaaggtatt 480
tcatcggttg aaagggcaca aaaagtccaa cccatgatga atcatgccag aaaggaaatt 540
ggagttgagg aagctattga ttacctaaag tctatcaatg ctccgttttg gaaaaatttt 600
gatggtagag gtatggcat ttcaaatac gatactggaa cagattatag acataaggct 660
atgagaatcg atgatgatc caaagcctca atgagattta aaaaagaaga cttaaaaggc 720
actgataaaa attattggtt gagtgataaa atccctcatg cgttcaatta ttataatggt 780
ggcaaaatca ctgtagaaaa atatgatgat ggaagggtatt attttgaccc acatgggatg 840
catattgcag ggattcttgc tggaaatgat actgaacaag acatcaaaaa ctttaacggc 900
atagatggaa ttgcacctaa tgcacaaatt ttctcttaca aaatgtattc tgacgcagga 960
tctgggtttg cgggtgatga aacaatgttt catgctattg aagattctat caaacacaac 1020
gttgatgttg tttcgggtatc atctggtttt acaggaacag gtctttagtag tgagaaatat 1080
tggcaagcta ttcgggcatt aagaaaagca ggcattccaa tggttgtcgc tacgggtaac 1140

tatgcgactt ctgcttcaag ttcttcatgg gatttagtag caaataatca tctgaaaatg 1200
 accgacactg gaaatgtaac acgaactgca gcacatgaag atgcgatagc ggtcgcttct 1260
 gctaaaaatc aaacagttga gtttgataaa gttaacatag gtggagaaag ttttaaatac 1320
 agaaatatag gggccttttt cgataagagt aaaatcacia caaatgaaga tggaacaaaa 1380
 gctcctagta aattaaaatt tgtatatata ggcaaggggc aagaccaaga tttgataggt 1440
 ttggatctta ggggcaaaat tgcagtaatg gatagaattt atacaaagga tttaaaaaat 1500
 gcttttaaaa aagctatgga taaggggtgca cgcgccatta tggttgtaa tactgtaaat 1560
 tactacaata gagataattg gacagagctt ccagctatgg gatatgaagc ggatgaaggt 1620
 actaaaagtc aagtgttttc aatttcagga gatgatgggtg taaagctatg gaacatgatt 1680
 aatcctgata aaaaaactga agtcaaaaga aataataaag aagattttta agataaattg 1740
 gagcaatact atccaattga tatggaaagt ttttaattcca acaaaccgaa tgtaggtgac 1800
 gaaaaagaga ttgacttta gtttgcacct gacacagaca aagaactcta taaagaagat 1860
 atcatcgttc cagcaggatc tacatcttgg gggccaagaa tagatttact tttaaaaccc 1920
 gatgtttcag cacctggtaa aaatattaaa tccacgctta atgttattaa tggcaaatca 1980
 acttatggct at 1992

<210> 6

<211> 664

<212> PRT

<213> Streptococcus pneumoniae

<400> 6

Tyr Pro Val Val Leu Ala Asp Thr Ser Ser Ser Glu Asp Ala Leu Asn

1 5 10 15

Ile Ser Asp Lys Glu Lys Val Ala Glu Asn Lys Glu Lys His Glu Asn

20 25 30

Ile His Ser Ala Met Glu Thr Ser Gln Asp Phe Lys Glu Lys Lys Thr

35 40 45

Ala Val Ile Lys Glu Lys Glu Val Val Ser Lys Asn Pro Val Ile Asp

50 55 60

Asn Asn Thr Ser Asn Glu Glu Ala Lys Ile Lys Glu Glu Asn Ser Asn

65 70 75 80

Lys Ser Gln Gly Asp Tyr Thr Asp Ser Phe Val Asn Lys Asn Thr Glu

85 90 95

Asn Pro Lys Lys Glu Asp Lys Val Val Tyr Ile Ala Glu Phe Lys Asp

100 105 110

Lys Glu Ser Gly Glu Lys Ala Ile Lys Glu Leu Ser Ser Leu Lys Asn

115 120 125

Thr Lys Val Leu Tyr Thr Tyr Asp Arg Ile Phe Asn Gly Ser Ala Ile

130	135	140
Glu Thr Thr Pro Asp Asn Leu Asp Lys Ile Lys Gln Ile Glu Gly Ile		
145	150	155 160
Ser Ser Val Glu Arg Ala Gln Lys Val Gln Pro Met Met Asn His Ala		
165	170	175
Arg Lys Glu Ile Gly Val Glu Glu Ala Ile Asp Tyr Leu Lys Ser Ile		
180	185	190
Asn Ala Pro Phe Gly Lys Asn Phe Asp Gly Arg Gly Met Val Ile Ser		
195	200	205
Asn Ile Asp Thr Gly Thr Asp Tyr Arg His Lys Ala Met Arg Ile Asp		
210	215	220
Asp Asp Ala Lys Ala Ser Met Arg Phe Lys Lys Glu Asp Leu Lys Gly		
225	230	235 240
Thr Asp Lys Asn Tyr Trp Leu Ser Asp Lys Ile Pro His Ala Phe Asn		
245	250	255
Tyr Tyr Asn Gly Gly Lys Ile Thr Val Glu Lys Tyr Asp Asp Gly Arg		
260	265	270
Asp Tyr Phe Asp Pro His Gly Met His Ile Ala Gly Ile Leu Ala Gly		
275	280	285
Asn Asp Thr Glu Gln Asp Ile Lys Asn Phe Asn Gly Ile Asp Gly Ile		
290	295	300
Ala Pro Asn Ala Gln Ile Phe Ser Tyr Lys Met Tyr Ser Asp Ala Gly		
305	310	315 320
Ser Gly Phe Ala Gly Asp Glu Thr Met Phe His Ala Ile Glu Asp Ser		
325	330	335
Ile Lys His Asn Val Asp Val Val Ser Val Ser Ser Gly Phe Thr Gly		
340	345	350
Thr Gly Leu Val Gly Glu Lys Tyr Trp Gln Ala Ile Arg Ala Leu Arg		
355	360	365
Lys Ala Gly Ile Pro Met Val Val Ala Thr Gly Asn Tyr Ala Thr Ser		
370	375	380
Ala Ser Ser Ser Ser Trp Asp Leu Val Ala Asn Asn His Leu Lys Met		

385		390		395		400
Thr Asp Thr Gly Asn Val Thr Arg Thr Ala Ala His Glu Asp Ala Ile						
	405		410		415	
Ala Val Ala Ser Ala Lys Asn Gln Thr Val Glu Phe Asp Lys Val Asn						
	420		425		430	
Ile Gly Gly Glu Ser Phe Lys Tyr Arg Asn Ile Gly Ala Phe Phe Asp						
	435		440		445	
Lys Ser Lys Ile Thr Thr Asn Glu Asp Gly Thr Lys Ala Pro Ser Lys						
	450		455		460	
Leu Lys Phe Val Tyr Ile Gly Lys Gly Gln Asp Gln Asp Leu Ile Gly						
465		470		475		480
Leu Asp Leu Arg Gly Lys Ile Ala Val Met Asp Arg Ile Tyr Thr Lys						
	485		490		495	
Asp Leu Lys Asn Ala Phe Lys Lys Ala Met Asp Lys Gly Ala Arg Ala						
	500		505		510	
Ile Met Val Val Asn Thr Val Asn Tyr Tyr Asn Arg Asp Asn Trp Thr						
	515		520		525	
Glu Leu Pro Ala Met Gly Tyr Glu Ala Asp Glu Gly Thr Lys Ser Gln						
	530		535		540	
Val Phe Ser Ile Ser Gly Asp Asp Gly Val Lys Leu Trp Asn Met Ile						
545		550		555		560
Asn Pro Asp Lys Lys Thr Glu Val Lys Arg Asn Asn Lys Glu Asp Phe						
	565		570		575	
Lys Asp Lys Leu Glu Gln Tyr Tyr Pro Ile Asp Met Glu Ser Phe Asn						
	580		585		590	
Ser Asn Lys Pro Asn Val Gly Asp Glu Lys Glu Ile Asp Phe Lys Phe						
	595		600		605	
Ala Pro Asp Thr Asp Lys Glu Leu Tyr Lys Glu Asp Ile Ile Val Pro						
	610		615		620	
Ala Gly Ser Thr Ser Trp Gly Pro Arg Ile Asp Leu Leu Leu Lys Pro						
625		630		635		640
Asp Val Ser Ala Pro Gly Lys Asn Ile Lys Ser Thr Leu Asn Val Ile						

Asn Gly Lys Ser Thr Tyr Gly Tyr
660

<210> 7

<211> 2319

<212> DNA

<213> Streptococcus pneumoniae

<400> 7

```

aagcttggcg agattgcaga atctaaattht aaaaatttag gaaatggaaa agagggtagt 60
ctaaaaaaag atacaactgg ggtagaacat catcatcaag aaaatgaaga gtctattaaa 120
gaaaaatcta gttttactat tgatagaaat atttcaacaa ttagagactt tgaaaaataaa 180
gacttaaaga aactcattaa aaagaaattht agagaagttg atgattttac aagtgaact 240
ggtaagagaa tggaggaata cgattataaa tacgatgata aaggaaatat aatagcctac 300
gatgatggga ctgatctaga atatgaaact gagaaacttg acgaaatcaa atcaaaaatt 360
tatggtgttc taagtccgtc taaagatgga cactttgaaa ttcttggaaa gataagtaat 420
gttttctaaaa atgccaaggt atattatggg aataactata aatctataga aatcaaagcg 480
accaagtatg atttccactc aaaaacgatg acatttgatc tatacgctaa tattaatgat 540
attgtggatg gattagcttt tgcaggagat atgagattat ttgttaaaga taatgatcag 600
aaaaaagctg aaattaaaaat tagaatgcct gaaaaaatta aggaaactaa atcagaatat 660
ccctatgtat caagttatgg gaatgtcata gaattagggg aaggagatct ttcaaaaaaac 720
aaaccagaca atttaactaa aatggaatct ggtaaaatct attctgattc agaaaaacaa 780
caatatctgt taaaggataa tatcattcta agaaaaggct atgcactaaa agtgactacc 840
tataatcctg gaaaaacgga tatgttagaa ggaaatggag tctatagcaa ggaagatata 900
gcaaaaaatac aaaaggccaa tcctaattcta agagcccttt cagaaacaac aatttatgct 960
gatagtagaa atgttgaaga tggaagaagt acccaatctg tattaatgtc ggctttggac 1020
ggctttaaca ttataaggta tcaagtgttt acatttaaaa tgaacgataa aggggaagct 1080
atcgataaag acggaaatct tgtgacagat tcttctaaac ttgtattatt tggtaaggat 1140
gataaagaat aactggaga ggataagttc aatgtagaag ctataaaaga agatggctcc 1200
atgttattta ttgataccaa accagtaaac ctttcaatgg ataagaacta ctttaatcca 1260
tctaaatcta ataaaattta tgtacgaaat ccagaattht atttaagagg taagatttct 1320
gataaggggtg gttttaactg ggaattgaga gttaatgaat cggttgtaga taattattta 1380
atctacggag atttacacat tgataacact agagatttht atattaagct gaatgttaaa 1440
gacggtgaca tcatggactg gggaatgaaa gactataaag caaacggatt tccagataag 1500
gtaacagata tggatggaaa tgtttatctt caaactggct atagcgattt gaatgctaaa 1560
gcagttggag tccactatca gtttttatat gataatgtta aaccgaagt aaacattgat 1620
cctaagggaa atactagtat cgaatatgct gatggaaaat ctgtagtctt taacatcaat 1680
gataaaagaa ataattggatt cgatggtgag attcaagaac aacatattta tataaatgga 1740
aaagaatata catcatttht tgatattaaa caaataatag acaagacact aaacattaag 1800
attgttgtta aagattttgc aagaaatata accgtaaaag aattcatttht aaataaagat 1860
acgggagagg taagtgaatt aaaacctcat agggtaactg tgaccattca aaatggaaaa 1920
gaaatgagtt caacgatagt gtcggaagaa gattttattt tacctgttta taagggtgaa 1980
ttagaaaaag gataccaatt tgatggttgg gaaatttctg gtttcgaagg taaaaaagac 2040
gctggctatg ttattaatct atcaaaagat acctttataa aacctgtatt caagaaaata 2100
gaggagaaaa aggaggaaga aaataaacct acttttgatg tatcgaaaaa gaaagataac 2160

```

ccacaagtaa accatagtca attaaatgaa agtcacagaa aagaggattt acaaagagaa 2220
gagcattcac aaaaatctga ttcaactaag gatgttacag ctacagttct tgataaaaac' 2280
aatatcagta gtaaatacaac tactaacaat cctaataag 2319

<210> 8

<211> 773

<212> PRT

<213> Streptococcus pneumoniae

<400> 8

Lys Leu Gly Glu Ile Ala Glu Ser Lys Phe Lys Asn Leu Gly Asn Gly
1 5 10 15

Lys Glu Gly Ser Leu Lys Lys Asp Thr Thr Gly Val Glu His His His
20 25 30

Gln Glu Asn Glu Glu Ser Ile Lys Glu Lys Ser Ser Phe Thr Ile Asp
35 40 45

Arg Asn Ile Ser Thr Ile Arg Asp Phe Glu Asn Lys Asp Leu Lys Lys
50 55 60

Leu Ile Lys Lys Lys Phe Arg Glu Val Asp Asp Phe Thr Ser Glu Thr
65 70 75 80

Gly Lys Arg Met Glu Glu Tyr Asp Tyr Lys Tyr Asp Asp Lys Gly Asn
85 90 95

Ile Ile Ala Tyr Asp Asp Gly Thr Asp Leu Glu Tyr Glu Thr Glu Lys
100 105 110

Leu Asp Glu Ile Lys Ser Lys Ile Tyr Gly Val Leu Ser Pro Ser Lys
115 120 125

Asp Gly His Phe Glu Ile Leu Gly Lys Ile Ser Asn Val Ser Lys Asn
130 135 140

Ala Lys Val Tyr Tyr Gly Asn Asn Tyr Lys Ser Ile Glu Ile Lys Ala
145 150 155 160

Thr Lys Tyr Asp Phe His Ser Lys Thr Met Thr Phe Asp Leu Tyr Ala
165 170 175

Asn Ile Asn Asp Ile Val Asp Gly Leu Ala Phe Ala Gly Asp Met Arg
180 185 190

Leu Phe Val Lys Asp Asn Asp Gln Lys Lys Ala Glu Ile Lys Ile Arg

195	200	205
Met Pro Glu Lys Ile Lys Glu Thr Lys Ser Glu Tyr Pro Tyr Val Ser 210 215 220		
Ser Tyr Gly Asn Val Ile Glu Leu Gly Glu Gly Asp Leu Ser Lys Asn 225 230 235 240		
Lys Pro Asp Asn Leu Thr Lys Met Glu Ser Gly Lys Ile Tyr Ser Asp 245 250 255		
Ser Glu Lys Gln Gln Tyr Leu Leu Lys Asp Asn Ile Ile Leu Arg Lys 260 265 270		
Gly Tyr Ala Leu Lys Val Thr Thr Tyr Asn Pro Gly Lys Thr Asp Met 275 280 285		
Leu Glu Gly Asn Gly Val Tyr Ser Lys Glu Asp Ile Ala Lys Ile Gln 290 295 300		
Lys Ala Asn Pro Asn Leu Arg Ala Leu Ser Glu Thr Thr Ile Tyr Ala 305 310 315 320		
Asp Ser Arg Asn Val Glu Asp Gly Arg Ser Thr Gln Ser Val Leu Met 325 330 335		
Ser Ala Leu Asp Gly Phe Asn Ile Ile Arg Tyr Gln Val Phe Thr Phe 340 345 350		
Lys Met Asn Asp Lys Gly Glu Ala Ile Asp Lys Asp Gly Asn Leu Val 355 360 365		
Thr Asp Ser Ser Lys Leu Val Leu Phe Gly Lys Asp Asp Lys Glu Tyr 370 375 380		
Thr Gly Glu Asp Lys Phe Asn Val Glu Ala Ile Lys Glu Asp Gly Ser 385 390 395 400		
Met Leu Phe Ile Asp Thr Lys Pro Val Asn Leu Ser Met Asp Lys Asn 405 410 415		
Tyr Phe Asn Pro Ser Lys Ser Asn Lys Ile Tyr Val Arg Asn Pro Glu 420 425 430		
Phe Tyr Leu Arg Gly Lys Ile Ser Asp Lys Gly Gly Phe Asn Trp Glu 435 440 445		
Leu Arg Val Asn Glu Ser Val Val Asp Asn Tyr Leu Ile Tyr Gly Asp		

450	455	460
Leu His Ile Asp Asn Thr Arg Asp Phe Asn Ile Lys Leu Asn Val Lys		
465	470	475 480
Asp Gly Asp Ile Met Asp Trp Gly Met Lys Asp Tyr Lys Ala Asn Gly		
485	490	495
Phe Pro Asp Lys Val Thr Asp Met Asp Gly Asn Val Tyr Leu Gln Thr		
500	505	510
Gly Tyr Ser Asp Leu Asn Ala Lys Ala Val Gly Val His Tyr Gln Phe		
515	520	525
Leu Tyr Asp Asn Val Lys Pro Glu Val Asn Ile Asp Pro Lys Gly Asn		
530	535	540
Thr Ser Ile Glu Tyr Ala Asp Gly Lys Ser Val Val Phe Asn Ile Asn		
545	550	555 560
Asp Lys Arg Asn Asn Gly Phe Asp Gly Glu Ile Gln Glu Gln His Ile		
565	570	575
Tyr Ile Asn Gly Lys Glu Tyr Thr Ser Phe Asn Asp Ile Lys Gln Ile		
580	585	590
Ile Asp Lys Thr Leu Asn Ile Lys Ile Val Val Lys Asp Phe Ala Arg		
595	600	605
Asn Thr Thr Val Lys Glu Phe Ile Leu Asn Lys Asp Thr Gly Glu Val		
610	615	620
Ser Glu Leu Lys Pro His Arg Val Thr Val Thr Ile Gln Asn Gly Lys		
625	630	635 640
Glu Met Ser Ser Thr Ile Val Ser Glu Glu Asp Phe Ile Leu Pro Val		
645	650	655
Tyr Lys Gly Glu Leu Glu Lys Gly Tyr Gln Phe Asp Gly Trp Glu Ile		
660	665	670
Ser Gly Phe Glu Gly Lys Lys Asp Ala Gly Tyr Val Ile Asn Leu Ser		
675	680	685
Lys Asp Thr Phe Ile Lys Pro Val Phe Lys Lys Ile Glu Glu Lys Lys		
690	695	700
Glu Glu Glu Asn Lys Pro Thr Phe Asp Val Ser Lys Lys Lys Asp Asn		

705		710		715		720									
Pro	Gln	Val	Asn	His	Ser	Gln	Leu	Asn	Glu	Ser	His	Arg	Lys	Glu	Asp
			725					730					735		
Leu	Gln	Arg	Glu	Glu	His	Ser	Gln	Lys	Ser	Asp	Ser	Thr	Lys	Asp	Val
			740					745					750		
Thr	Ala	Thr	Val	Leu	Asp	Lys	Asn	Asn	Ile	Ser	Ser	Lys	Ser	Thr	Thr
			755					760					765		
Asn	Asn	Pro	Asn	Lys											
			770												